Platform S: Protein Assemblies and Protein Nucleic Acid Complexes

1079-Plat
Ion Mobility-Mass Spectrometry Measurements Combined with Molecular Modeling Yields the Architecture of DNA Polymerase Complexes
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Understanding how DNA polymerases interact with the rest of the replication machinery is of primary importance in unraveling some long-standing questions surrounding the mechanisms of DNA replication. We have been developing a method that combines both molecular and coarse-grained modeling and ion mobility-mass spectrometry (IM-MS) measurements in order to determine the positions of the protein subunits within the complex. The method works by first ionizing and desolvating the protein assemblies using nano-electrospray ionization. We then estimate the sizes of the protein ions by measuring their transit time through an IM separator, and measure their masses to determine their composition. To construct a model of the complex, MS information is used to infer subunit connectivity and IM information is used to filter the size of the assembly against a field of computationally derived model structures. Central to our method is the use of these measurements on various subcomplexes generated either in the solution or gas phases. When available, we use crystal structures of proteins and subcomplexes to build up models of complexes of unknown architecture, calculating and comparing their sizes with what is being measured by IM. Here, we present our most recent models for three different polymerases (II, III, and IV) bound to the prokaryotic DNA sliding clamp, the clamp loader bound to the clamp, and the chi-psi dimer bound to the clamp loader. The presentation will be evenly balanced between the development of this hybrid approach and its application to DNA polymerase III.

1080-Plat
Direct Measurement of Inter-filament Forces in Neurofilament Networks: Synchrotron X-ray Diffraction Study under Osmotic Pressure
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Neurofilaments (NFs) are the major protein constituents in neuronal processes (axons and dendrites) that impart mechanical stability and act as structural scaffolds. The filaments assemble from 3 different subunit proteins (NF-L, NF-M, NF-H) to form a 10 nm diameter flexible polymer with radiating unstructured sidearms. Recent work, showed that at high protein concentration, the NFs form a nematic hydrogel network with a well-defined interfilament spacing as can be measured by synchrotron small angle x-ray scattering (SAXS) [1]. The x-ray/phase behavior study showed the role the different subunit protein compositions play in the interfilament interaction. In order to directly elucidate the interfilament forces responsible for the mechanical properties of NFs hydrogel, we conducted a SAXS-osmotic pressure study, which yielded pressure-distance curves at different subunit compositions and monovalent salts. We show that filaments composed with NF-L and NF-M strongly attract each other through their polyampholyte sidearms, in particularly at high monovalent salt. However, filaments comprised of NF-L and NF-H, at high NF-H grafting density, show a distinctly different pressure-distance dependency, with much larger interfilament spacing and weaker salt dependence. Supported by DOE DE-FG-02-06ER46314, NIH GM-59288, NSF DMR-0503347, and the Human Frontier Science Program organization.
[1] J.B. Jones, C.R. Safinya, Biophys. J. 95, 823 (2008).

1081-Plat
DNA Heats Up: Energetics of Genome Ejection from Phage Revealed by Isothermal Titration Calorimetry
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It has been experimentally shown that ejection of double-stranded DNA from phage is driven by internal pressure reaching tens of atmospheres. This internal pressure is partially responsible for delivery of viral genome into the host cell. While several theoretical models and simulations nicely describe the experimental data of internal forces either resisting active packaging or equivalently favoring spontaneous ejection, there are no direct energy measurements available that would help to verify how quantitative these theories are. We performed direct measurements of the enthalpy (which is essentially equivalent to the internal energy) responsible for DNA ejection from phage λ, using Isothermal Titration Calorimetry (ITC). The phage capsids were ''opened'' in vitro by titrating λ particles into a solution with purified LamB receptor and the enthalpy of DNA ejection process was measured. In this way, enthalpy stored in phage was determined as a function of packaged DNA length comparing wild-type phage λ (48.5 kb) with a shorter λ-DNA length mutant (37.7 kb). The temperature dependence of the ejection enthalpy was also investigated. The values obtained were in good agreement with existing models and provide a better understanding of double-stranded DNA packaging and release mechanisms in motor-packaged viruses (e.g., tailed bacteriophages, Herpes Simplex, and adenoviruses).

1082-Plat
Nucleotide Control of Replication Initiation: NTPase Mechanisms of *E.coli* DnaB-DnaC Complex
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In E. coli, interactions between the primary replicative helicase, DnaB protein and the replication factor, DnaC protein, are at the heart of the chromosomal DNA replication. To address the role of the DnaC in processes of the free energy transduction by the helicase, we have quantitatively examined DnaB-DnaC complex using fluorescence titration, analytical ultracentrifugation, and rapid chemical quench-flow techniques.
In absence of nucleic acid, DnaC reduces intrinsic affinity and increases negative cooperativity in nucleotide binding to DnaB helicase. The ground-state effects are accompanied by the reduced rate of ATP hydrolysis by the helicase. In presence of DNA, DnaB in the DnaB-DnaC complex recovers its nucleotide binding capabilities and ATPase activity. These data suggest that recognition of the oriC by the DnaB - DnaC complex and/or its entry into the pre-primosome requires diminished NTPase activity of the helicase. Analysis of nucleotide binding to the DnaC protein, engaged in the DnaB - DnaC complex, indicates that *prior* to the recognition of the oriC sequence and/or pre-primosome assembly, the DnaC protein in the complex is in a conformational state, which does not bind ATP or ADP. So, the formation of the replisome and the pre-primosome seems to preferentially require the presence of DnaC in a state free of cofactors. Significant positive cooperativity of the binding process indicates that small fluctuations in ATP and/or ADP concentrations can induce an all-or-none allosteric transition of bound DnaC molecules into the conformation, which has an increased intrinsic affinity for the nucleotides. The presence of such an all-or-none allosteric transition, encompassing all bound DnaC molecules, indicates that recognition of the oriC and the pre-primosome assembly includes complex interplay between different conformations of the DnaB - DnaC complex. The physiological importance of the obtained results will be discussed.

1083-Plat
Oligomerization And Interaction Of DDR1 With Collagen: An AFM And FRET Microscopy Study
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Activation of Discoidin Domain Receptor 1 (DDR1) by collagen is reported to regulate cell migration and survival processes. While the oligomeric state of DDR1 is reported to play a significant role in collagen binding, not much is known about the effect of collagen binding on DDR1 oligomerization and cellular distribution. Using fluorescence resonance energy transfer (FRET) microscopy we monitored the interaction between DDR1 tagged with either cyan (CFP) or yellow fluorescent protein (YFP) on live cells. Significant FRET signal, indicative of receptor dimerization was found even in the absence of collagen stimulation. Collagen stimulation induced aggregation of DDR1 followed by a sharp increase in FRET signal, localized in the regions of aggregated receptor. Further analysis of DDR1 aggregation revealed that DDR1 undergoes cytoplasmic internalization and incorporation into the early endosome. We found the kinetics of DDR1 internalization to be fast, with a significant percentage of the receptor population being internalized in the first few minutes of collagen stimulation. Our results indicate that collagen stimulation induces aggregation and internalization of DDR1 dimers at timescales much before receptor activation. These findings provide new insights in the cellular redistribution of DDR1 following its interaction with collagen type I.

1084-Plat
The Measured Electrostatic Charge on IgGs
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Blood plasma is a high-concentration fluid containing ~70 mg/ml protein. A major component of plasma (~10 mg/ml) is a changing, heterogeneous mixture of IgGs. At plasma concentrations electrostatics (charge, dipole, induced